

(19)



Europäisches Patentamt

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Office européen des brevets



(11)

**EP 0 750 509 B1**

(12)

## EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention  
of the grant of the patent:  
**15.05.2002 Bulletin 2002/20**

(51) Int Cl.7: **A61K 38/20, A61K 39/395,  
A61P 37/00**

(21) Application number: **95912666.5**

(86) International application number:  
**PCT/US95/02550**

(22) Date of filing: **07.03.1995**

(87) International publication number:  
**WO 95/24918 (21.09.1995 Gazette 1995/40)**

(54) **USE OF IL-12 AND IL-12 ANTAGONISTS for the manufacture of a pharmaceutical composition  
for THE TREATMENT OF AUTOIMMUNE DISEASES**

**VERWENDUNG VON IL-12 UND IL-12 ANTAGONISTEN zur Herstellung eines Arzneimittels FÜR  
BEHANDLUNG VON AUTOIMMUNKRANKHEITEN**

**UTILISATION DE L'IL-12 ET DES ANTAGONISTES D'IL-12 pour la fabrication d'une composition  
pharmaceutique utile DANS LE TRAITEMENT DES MALADIES AUTO-IMMUNES**

(84) Designated Contracting States:  
**AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL  
PT SE**

(30) Priority: **14.03.1994 US 212629**

(43) Date of publication of application:  
**02.01.1997 Bulletin 1997/01**

(60) Divisional application:  
**01117762.3 / 1 179 348**

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**EP 0 750 509 B1**

## Description

### Background of the Invention

**[0001]** Gamma interferon (IFN- $\gamma$ ) and tumor necrosis factor-alpha (TNF- $\alpha$ ) have been implicated in the development, exacerbation and/or recurrence of numerous autoimmune conditions. For example, both IFN- $\gamma$  and TNF- $\alpha$  have been associated with the course of multiple sclerosis [Choflon et al., Eur. Cytokine Netw. 3(6), 1992, pp. 523-531; Steinman, Scientific American, September 1993, pp. 107-114; Hofman et al., J. Exp. Med. 170, 1989, pp. 607-612; Panitch et al., Neurology, 37, 1987, pp. 1097-1102] and Type-I diabetes (insulin-dependent diabetes mellitus, IDDM) [Castano et al., Annu. Rev. Immunol. 8, 1990, pp. 647-679; Campbell et al., J. Clin. Invest. 87, 1991, pp. 739-742]. While TNF- $\alpha$  has been found to promote development of rheumatoid arthritis [Feldmann et al., Progress in Growth Factor Research, 4, 1992, pp. 247-255], administration of IFN- $\gamma$  has been linked to improvements in arthritic subjects [Veys et al., J. Rheumatology, 15(4), 1988, pp. 570-574]. Studies have also demonstrated the involvement of IFN- $\gamma$  in the autoimmune diseases processes associated with systemic lupus erythematosus (SLE) [Funauchi et al., Tohoku J. Exp. Med., 164, 1991, pp. 259-267; Bankhurst, J. Rheumatology, 14(supp. 13), 1987, pp. 63-67], autoimmune thyroiditis [Tang et al., Eur. J. Immunol. 23, 1993, pp. 275-278], and autoimmune inflammatory eye disease (e.g., autoimmune uveoretinitis) [Charteris et al., Immunology 75, 1992, pp. 463-467]. Development of autoimmune pulmonary inflammation [Deguchi et al., Clin. Exp. Immunol. 85, 1991, pp. 392-395] and Guillain-Barre syndrome [Baron et al., Proc. Natl. Acad. Sci. USA 90, 1993, pp. 4414-4418] have also been tied to TNF- $\alpha$  activity.

**[0002]** Interleukin-12 (IL-12) is a heterodimeric cytokine which was originally identified as a factor which induces IFN- $\gamma$  from T cells and natural killer cells as set forth in PCT/US91/06332, published April 2, 1992. PCT/US91/06332 refers to IL-12 as Natural Killer Cell Stimulating Factor or NKSF. EP 433827, published June 26, 1991 discloses IL-12 as a cytotoxic lymphocyte maturation factor (CLMF). IL-12 also stimulates natural killer cells *in vitro* by increasing their ability to lyse target cells at a level comparable to that obtained with IFN- $\alpha$  and IL-2, well-known activators of natural killer cells' cytotoxic activity. Additional *in vitro* activities of IL-12 which have been identified include induction of TNF- $\alpha$ ; induction of T cell proliferation as a co-stimulant; suppression of IL-2 induced proliferation of natural killer blasts; suppression of IL-2 induced proliferation of T cell receptor- $\gamma\delta$ -positive cells; promotion of Th1 T cell differentiation from progenitors; enhancement of Th1, but not Th2 proliferation; enhancement of T cell cytolytic activity; enhancement of cytotoxic lymphocyte generation; enhancement of natural killer and natural killer blast cytolytic activity; *ex vivo* enhancement of natural killer activity in peripheral blood mononuclear cells of IL-2-treated patients; induction of adhesion molecules on natural killer cells; induction of perforin and granzyme B mRNAs in natural killer blasts; induction of IL-2 receptor subunits (p55, p75) on natural killer cells; suppression of IgE synthesis by IFN- $\gamma$ -dependent and independent mechanisms; modulation of T cell development in fetal thymic organ cultures; and synergy with kit ligand to promote growth of myeloid and B cell progenitors. The known *in vivo* activities of IL-12 include induction of IFN- $\gamma$ ; enhancement of natural killer cell activity in spleen, liver, lungs and peritoneal cavity; enhancement of generation of allo-specific cytotoxic lymphocytes; induction of extramedullary hematopoiesis in mouse spleen; reversible suppression of hematopoiesis in bone marrow; reversible induction of anemia, lymphopenia, and neutropenia in mice; suppression of anti-IgD induced IgE, IgG1, and IL-4 expression; increased survival in SCID mice treated with *Toxoplasma gondii*; cure of leishmaniasis in susceptible strains of mice; decreased bioburden in cryptococcosis model; suppression of tumor growth; and promotion of immunity to tumor cells. IL-12 is also induced *in vivo* in the Schwartzman reaction model of septic shock.

**[0003]** Although IL-12 can induce production of IFN- $\gamma$  and TNF- $\alpha$  *in vivo*, the relationship of *in vivo* levels of IL-12 to autoimmune diseases which are affected by levels of IFN- $\gamma$  and TNF- $\alpha$  has not been established. Furthermore, the effects of administration of IL-12 or antagonists of endogenous IL-12 (such as anti-IL-12 antibodies) on autoimmune diseases associated with induction of IFN- $\gamma$  or TNF- $\alpha$  have not been examined.

### Summary of the Invention

**[0004]** The present invention provides use of an IL-12 antagonist for the preparation of a pharmaceutical composition for treating (e.g., curing, ameliorating, delaying or preventing onset of, preventing recurrence or relapse of) autoimmune conditions or diseases. In preferred embodiments, the condition is one promoted by an increase in levels of a cytokine selected from the group consisting of TNF- $\alpha$  or IFN- $\gamma$ . Such conditions include, without limitation, those selected from the group consisting of multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus and autoimmune inflammatory eye disease. Multiple sclerosis and insulin-dependent diabetes mellitus are particularly preferred conditions for treatment in accordance with the present invention as described herein.

**[0005]** In certain embodiments the use for the treatment of the present invention comprises administering to a mammalian subject a therapeutically effective amount of an IL-12 antagonist, preferably an antibody or other species which

is immunoreactive with IL-12. In certain preferred embodiments the IL-12 antagonist is administered in a dose of about 0.05 to about 25 mg/kg, preferably of about 0.2 to about 2 mg/kg. The antagonists can also be administered in combination with a pharmaceutically acceptable carrier.

[0006] In other embodiments, the use of the present invention comprises administering to a mammalian subject a therapeutically effective amount of IL-12. In certain embodiments, the IL-12 may be administered in a dose of about 0.001 to about 1000  $\mu\text{g/kg}$ , preferably about 0.01 to about 100  $\mu\text{g/kg}$ . The IL-12 can also be administered in combination with a pharmaceutically acceptable carrier.

#### Brief Description of the Figures

[0007] Fig. 1 presents graphs of data relating to the adoptive transfer of experimental allergic encephalomyelitis (EAE) using lymph node and spleen cells stimulated *in vitro* with PLP and rmlL-12. Spleens and Lymph nodes were harvested from mice 10 days after immunization with PLP and stimulated *in vitro* with antigen alone (open symbols) or antigen and 20ng/ml rmlL-12 (closed symbols) as described in materials and methods. Disease was transferred using  $30 \times 10^6$  cells. The results are presented as mean score for (a) lymph nodes (n=7) and (b) spleen cells (n=5). The data is representative of at least two separate experiments. See example 1.

[0008] Fig. 2 presents graphs of data relating to IFN- $\gamma$  and TNF- $\alpha$  production from LNC stimulated *in vitro* with PLP and IL-12. LNC ( $2.5 \times 10^6/\text{ml}$ ) from PLP immunized mice were cultured with PLP alone, PLP and rmlL-12 (20ng/ml) or PLP, rmlL-12 and anti-IFN- $\gamma$  (5 $\mu\text{g/ml}$ ) for 96 hours prior to cell transfer with  $30 \times 10^6$  cells. (a) IFN- $\gamma$  and TNF- $\alpha$  measured by ELISA in the supernatants of pooled cultures. (b) Mean disease score after the transfer of stimulated lymph node cells. n=3 for PLP alone and PLP + IL-12 and n=4 for PLP + IL-12 + anti-IFN- $\gamma$ . See example 1.

[0009] Fig. 3 depicts graphs of data relating to the effects of *In vivo* administration of IL-12 on the adoptive transfer of EAE using PLP stimulated LNC. LNC from PLP immunized mice were cultured *in vitro* with antigen as described in materials and methods and transferred to naive mice. rmlL-12 (0.3 $\mu\text{g/mouse}$ ) was administered on days 0, 1 and 2 after cell transfer (closed circles) and mice monitored for signs of disease. Control mice received an equal volume of saline (open circles). (a) Mean clinical score following the transfer of  $30 \times 10^6$  LNC cells (n=5). (b) Mean clinical score following the transfer of  $10 \times 10^6$  LNC (n=4). Figure 3a is representative of three separate experiments. See example 1.

[0010] Fig. 4 depicts graphs of data relating to the effects of *in vivo* administration of anti-IL-12 antibody on the adoptive transfer of EAE using PLP stimulated LNC. LNC from PLP immunized mice were cultured *in vitro* with antigen as described in materials and methods and  $30 \times 10^6$  cells transferred to naive mice. Anti-IL-12 antibody (sheep anti-mouse polyclonal antibody, 200 $\mu\text{g/ mouse}$ ) was administered by intraperitoneal injection starting on the day of cell transfer (closed circles). Control mice received an equivalent amount of sheep IgG (open circles). (a) Mean clinical score following administration of  $\alpha\text{IL-12}$  antibody every other day from day 0 to day 6. (b) Mean clinical score following administration of  $\alpha\text{IL-12}$  antibody every other day from day 0 to day 12. (n=5-7). See example 1.

[0011] Figs. 5 and 6 present graphs of data relating to disease incidence in NOD mice upon administration of IL-12. See example 2.

#### Detailed Description

[0012] The present invention provides use of IL-12 antagonist for the preparation of a pharmaceutical composition for treating autoimmune conditions. "Autoimmune conditions" are those in which the subject's own immune system reacts against the subject's cells or tissues, resulting in damage to those cells or tissues. A particular autoimmune condition is "promoted by an increase in levels of a cytokine" when an increase in serum or tissue levels of such cytokine can cause or contribute to the development or recurrence of, or to the acceleration of the onset of, such autoimmune condition. Autoimmune conditions which are promoted by an increase in levels of IFN- $\gamma$  and/or TNF- $\alpha$  include, without limitation, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus and autoimmune inflammatory eye disease.

[0013] "IL-12 antagonists" include (1) species that will bind IL-12 or biologically active fragments thereof, and (2) species that will interfere with the binding of IL-12 to receptors or other binding proteins. Antagonists that bind IL-12 include, without limitation, antibodies (mono- or polyclonal) and fragments thereof (including  $F_{ab}$  fragments), chimeric antibodies and fragments thereof, lectins, IL-12 receptors or fragments thereof, reactive peptides or fragments thereof, and organic small molecules designed to mimic the bioactivity of IL-12 receptors. Antagonists that interfere with IL-12 binding include, without limitation, chemically or genetically modified peptides of IL-12, subunits of IL-12 and fragments thereof, homopolymers of IL-12 subunits and fragments thereof, and organic small molecules designed to mimic the bioactivity of IL-12. Preferably, antagonists that interfere with IL-12 binding interfere with its binding to receptors which induce IFN- $\gamma$  or TNF- $\alpha$ , without inducing the same level of such factors as would binding of IL-12 to the receptor.

[0014] IL-12 antagonists can be produced by methods well known to those skilled in the art. For example, monoclonal

IL-12 antibodies can be produced by generation of antibody-producing hybridomas in accordance with known methods (see for example, Goding, 1983. *Monoclonal antibodies: principles and practice*. Academic Press Inc., New York; Yokoyama, 1992. "Production of Monoclonal Antibodies" in *Current Protocols in Immunology*. Unit 2.5. Greene Publishing Assoc. and John Wiley & Sons). Polyclonal sera and antibodies to IL-12 can be produced by inoculation of a mammalian subject with IL-12 or fragments thereof in accordance with known methods. Chizzonite et al., *J. Immunol.* 148, 1992, p. 3117, describes the identification and isolation of an IL-12 receptor. Fragments of antibodies, receptors or other reactive peptides can be produced from the corresponding antibodies by cleavage of and collection of the desired fragments in accordance with known methods (see for example, Goding, supra; Andrew et al. 1992. "Fragmentation of Immunoglobulins" in *Current Protocols in Immunology*. Unit 2.8. Greene Publishing Assoc. and John Wiley & Sons). Chimeric antibodies may also be produced in accordance with known methods.

**[0015]** In uses of the present invention using IL-12, any form of IL-12 may be used, so long as that form of IL-12 is capable of treating the desired autoimmune condition. For example, IL-12 may be in the form of the heterodimer comprised of a 40 kD subunit disulfide-bonded to a 35 kD subunit. When IL-12 is a heterodimer, the 40 kD subunit has substantial homology to the 40 kD subunit of human IL-12 as set forth in PCT/US91/06332 and is disulfide bonded to a 35 kD subunit having substantial homology to the 35 kD subunit of human IL-12 as set forth in that same PCT publication. "Substantial homology" means greater than 75% homology at the amino acid level, while retaining the ability to treat the desired autoimmune condition in a mammalian subject. Another form of IL-12 which may be used in the present invention is an IL-12 subunit capable of treating the desired autoimmune condition in a mammalian subject. Such an IL-12 40 kD subunit has substantial homology to the human IL-12 40 kD subunit disclosed in PCT/US91/06332, and such an IL-12 35 kD subunit has substantial homology to the human IL-12 35 kD subunit disclosed in such PCT publication. Fragments of the IL-12 subunits that retain IL-12 biological activity are also be useful to treat autoimmune conditions in mammalian subjects, in accordance with the present invention.

**[0016]** For use in the present invention, it is preferable to produce IL-12 recombinantly, through expression of DNA sequences encoding one or both of the IL-12 subunits in a suitable transformed host cell. For example, using known methods the DNA sequences encoding human IL-12 set forth in PCT/US91/06332 may be linked to an expression vector such as pED (Kaufman et al., *Nucleic Acids Res.* 19, 4484-4490(1991)). In such an expression vector, sequences which optimize translation such as CCACC (Kozak, M., *Nucleic Acids Res.* 12, 857-871 (1984)) may be added 5' to the initiation codon using known methods. The expression vector containing the IL-12 subunits may then be transformed into a host cell, and protein expression may be induced and maximized, to produce heterodimeric human IL-12. For production of heterodimeric IL-12, the DNA sequences encoding the IL-12 subunits may be present on different expression plasmids or present in tandem on a single expression plasmid.

**[0017]** When a subunit or fragment of IL-12 is used to practice the present invention, it may also be produced recombinantly using known methods. For example, the DNA sequence encoding the human IL-12 40 kD subunit set forth in PCT/US91/06332 may be linked to an expression vector, transformed into a host cell, and expression induced and maximized to produce the human IL-12 40 kD subunit. Similarly, the DNA sequences encoding the human IL-12 35 kD subunit as set forth in the PCT publication may be linked to an expression vector, transformed into a host cell, and expression induced and maximized to produce the corresponding protein. Of course, degenerate DNA sequences encoding the IL-12 subunits may also be employed to produce IL-12 for use in the present invention, as can DNA sequences encoding allelic variants of the IL-12 subunits. Chemically or genetically modified forms of IL-12 and its subunits can also be made in accordance with the methods disclosed in the PCT publication.

**[0018]** Any suitable expression vector may be employed to produce IL-12 for use in the present invention. For mammalian expression, numerous expression vectors are known in addition to the pED vector mentioned above, such as pEF-BOS (Mizushima et al., *Nucleic Acids Res.* 18, 5322 (1990)); pXM, pJL3 and pJL4 (Gough et al., *EMBO J.* 4, 645-653 (1985)); and pMT2 (derived from pMT2-VWF, A.T.C.C. #67122; see PCT/US87/00033). Suitable expression vectors for use in yeast, insect, and bacterial cells are also known. Construction and use of such expression vectors is well within the level of skill in the art.

**[0019]** Suitable host cells for recombinant production of IL-12 useful in the present invention include, for example, mammalian cells such as Chinese hamster ovary (CHO) cells, monkey COS cells, mouse 3T3 cells, mouse L cells, myeloma cells such as NSO (Galfre and Milstein, *Methods in Enzymology* 73, 3-46 (1981)), baby hamster kidney cells, and the like. IL-12 may also be produced by transformation of yeast, insect, and bacterial cells with DNA sequences encoding the IL-12 subunits, induction and amplification of protein expression, using known methods.

**[0020]** Recombinantly produced IL-12 can be purified from culture medium or cell extracts by conventional purification techniques. Culture medium or cell extracts containing IL-12 may be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration medium. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices

comprising sulfopropyl or carboxymethyl groups. The purification of IL-12 from culture supernatant may also include one or more column steps over such affinity resins as lectin-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; or by hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or by immunoaffinity chromatography. Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify IL-12 for use in the present methods and compositions. Some or all of the foregoing purification steps, in various combinations, can be employed to provide a substantially homogeneous isolated recombinant protein. Purification of IL-12 subunits or fragments for use in the present invention may differ from the optimal protocol for purification of the heterodimeric protein.

**[0021]** Preferably, when human IL-12 is produced recombinantly as set forth above, it may be purified by the following method. The cells in which the human IL-12 has been made may be removed from the conditioned medium by filtration, and the conditioned medium is loaded onto Q-Sepharose FastFlow™ (available from Pharmacia) or an equivalent anion exchange medium, which has been equilibrated in 10-30 mM Tris-HCl, pH 7.8-8.3. The column is then washed extensively with the same buffer followed by a wash with 30-45 mM histidine, pH 5.1-5.8, followed by a wash with the original equilibration buffer. The recombinant human IL-12 is eluted from the column with a buffer containing 20-50 mM Tris-HCl, pH 7.8-8.5, and 0.15 to 0.50 M NaCl. The eluted material is loaded onto CM-Sepharose FastFlow™ (available from Pharmacia) or equivalent cation exchange medium which has been equilibrated in 20-50 mM MES, pH 5.7-6.4, and washed extensively with the same buffer. The column is washed with a buffer containing 20-40 mM sodium phosphate, pH 6.8-7.5 and 0.2-0.5 M NaCl. The eluted material is concentrated using an Amicon™ S1Y30 or equivalent spiral cartridge membrane which has been washed and equilibrated in the elution buffer used in the CM-Sepharose FastFlow™ column. The material is concentrated to approximately 5% of the column volume of the final chromatographic step, which is size exclusion using S200 Sephacryl™ (available from Pharmacia) or an equivalent size exclusion resin. The size exclusion column is equilibrated and eluted with phosphate buffered saline, pH 7.2-7, and the recombinant human IL-12 peak is collected and filtered for use in the method of the invention. Those of skill in the art of protein purification may use alternative purification methods to obtain recombinantly-produced human IL-12 for use in the method of the invention.

**[0022]** IL-12 may be purified from culture medium or extracts of cells which naturally produce the protein and used in the present invention. Exemplary purification schemes for naturally produced IL-12 are set forth in PCT/US91/06332 and in EP 433827.

**[0023]** Pharmaceutical compositions containing an IL-12 antagonist or IL-12 which are useful in the uses of the present invention may also contain pharmaceutically acceptable carriers, diluents, fillers, salts, buffers, stabilizers and/or other materials well-known in the art. The term "pharmaceutically acceptable" means a material that does not interfere with the effectiveness of the biological activity of the active ingredient(s) and that is not toxic to the host to which it is administered. The characteristics of the carrier or other material will depend on the route of administration.

**[0024]** It is currently contemplated that the various pharmaceutical compositions should contain about 0.1 micrograms to about 1 milligram per milliliter of the IL-12 antagonist or IL-12.

**[0025]** Administration can be carried out in a variety of conventional ways. Intraperitoneal injection is the preferred method of administration of the IL-12 antagonist or IL-12. Intravenous, cutaneous or sub-cutaneous injection may also be employed. For injection, IL-12 antagonist or IL-12 will preferably be administered in the form of pyrogen-free, parenterally acceptable aqueous solutions. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability and the like, is within the skill of the art.

**[0026]** The amount of IL-12 antagonist or IL-12 used for treatment will depend upon the severity of the condition, the route of administration, the reactivity of the IL-12 antagonist with IL-12 or the activity of the IL-12, and ultimately will be decided by the treatment provider. In practicing the methods of treatment of this invention, a therapeutically effective amount of an IL-12 antagonist or IL-12 is administered. The term "therapeutically effective amount" means the total amount of each active component of the method or composition that is sufficient to show a meaningful patient benefit (e.g., curing, ameliorating, delaying or preventing onset of, preventing recurrence or relapse of). One common technique to determine a therapeutically effective amount for a given patient is to administer escalating doses periodically until a meaningful patient benefit is observed by the treatment provider. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously. A therapeutically effective dose of an IL-12 antagonist in this invention is contemplated to be in the range of about 0.05 mg/kg to about 25 mg/kg. A therapeutically effective dose of IL-12 in this invention is contemplated to be in the range of about 0.001 to about 1000 µg/kg. The number of administrations may vary, depending on the individual patient and the severity of the autoimmune condition.

**[0027]** The IL-12 antagonist or IL-12 used in practicing the present invention may be administered alone or combined with other therapies for autoimmune conditions, such as steroidal or other anti-inflammatory therapies and administration of other cytokines.

[0028] The uses of the present invention are further described in the following examples, which are intended to illustrate the invention.

#### Example 1

[0029] Experimental allergic encephalomyelitis (EAE) is a T cell mediated autoimmune disease of the central nervous system (CNS). Disease can be induced in susceptible strains of mice by immunization with CNS myelin antigens or alternatively, disease can be passively transferred to susceptible mice using antigen stimulated CD4<sup>+</sup> T cells [Pettinelli, J. Immunol. 127, 1981, p. 1420]. EAE is widely recognized as an acceptable animal model for multiple sclerosis in primates [Alvord et al. (eds.) 1984. Experimental allergic encephalomyelitis - A useful model for multiple sclerosis. Alan R. Liss, New York]. The effects of administration of an IL-12 antagonist on induction of EAE following the adoptive transfer of lymphocytes from immunized mice restimulated *in vitro* with a synthetic peptide of myelin proteolipid protein (PLP) were analyzed.

#### **Adoptive transfer of PLP sensitized LNC**

[0030] Female SJL/J mice (7-10wks) were purchased from The Jackson Laboratory, housed 5 to a cage and fed standard rodent chow diet with water ad libitum. Mice were immunized in two sites on the flank with 150µg of mouse PLP peptide comprising residues 139-151 (provided by G Brown, Genetics Institute). PLP was administered in 200µl of Complete Freund's adjuvant containing 2mg/ml *Mycobacteria Tuberculosis* H37RA (Difco). On the day of immunization mice were injected intravenously with  $0.75 \times 10^{10}$  *Bordetella pertussis* bacilli (Massachusetts Public Health Laboratories, Boston, MA). Ten days after immunization, spleens and lymph nodes (popliteal, axillary and brachial) were harvested and the cells resuspended in RPMI-1640 containing 10% FBS (Hyclone),  $5 \times 10^{-5}$  M 2-Mercaptoethanol, 100µg/ml streptomycin and 100U/ml penicillin. PLP was added to the cultures at 2µg/ml. After 96 hours, the cells were harvested, washed twice and  $30 \times 10^6$  cells (either LNC or spleen) injected i.p. into naive SJL/J mice.

#### **Clinical evaluation of disease**

[0031] Mice were observed for clinical signs of EAE and scored on a scale of 0 to 3 as follows:

- 0.5 - Distal limp tail
- 1.0 - Complete limp tail
- 1.5 - Limp tail and hind limb weakness (unsteady gait)
- 2.0 - Partial hind limb paralysis
- 3.0 - Complete bilateral hind limb paralysis

#### **In Vitro Administration of IL-12 prior to cell transfer.**

[0032] Recombinant murine IL-12 (20ng/ml, rmIL-12, Genetics Institute) was added to the *in vitro* cultures of lymph node or spleen cells with antigen prior to cell transfer. After 96 hours the cells were washed twice and  $30 \times 10^6$  cell transferred to naive SJL/J mice to determine the effects of IL-12 on the subsequent course of disease.

[0033] In separate experiments, LNC were cultured with either antigen alone, antigen plus IL-12 (20ng/ml) or antigen plus IL-12 plus a neutralizing antibody to IFN-γ (5µg/ml from Endogen). At the end of the culture period supernatants were collected (pooled from three flasks) and IFN-γ and TNF-α measured by ELISA (from Genzyme).  $30 \times 10^6$  cells from each group were transferred to naive mice which were monitored for signs of disease.

#### **In vivo Administration of IL-12 and Anti-IL-12 antibody following the transfer of PLP stimulated LNC**

[0034] rmIL-12 (0.3µg/mouse, 200µl i.p.) was administered to mice following the transfer of either  $30 \times 10^6$  or  $10 \times 10^6$  PLP stimulated LNC. IL-12 was administered on days 0, 1 and 2 following cell transfer. Control mice received an equal volume of vehicle alone. To determine if IL-12 is involved in the induction of disease following the transfer of PLP stimulated LNC, mice were treated with 200µg of a sheep polyclonal antibody against murine IL-12 (200µl i.p.) on alternate days for either 6 or 12 days in total following cell transfer and the mice monitored for signs of disease. Control mice received an equal amount of sheep IgG. The mice were monitored.

#### **Effect of rmIL-12 on restimulation of PLP primed T cells *in vitro***

[0035] LNC from mice immunized with PLP as described in methods were stimulated *in vitro* with antigen in the

absence or presence of mIL-12 (20ng/ml) for 96 hours after which time they were tested for their ability to transfer disease to naive SJL/J mice. Mice receiving LNC stimulated in vitro with PLP alone developed clinical signs of disease between days 6 and 8. All control mice reached scores of 2 or greater (7/7) with 4 out of 7 mice progressing to complete hind limb paralysis which lasted between 1 and 4 days (Fig. 1a). All the control mice had recovered by day 19. In contrast mice receiving cells cultured in vitro with PLP and IL-12 developed severe EAE with rapid onset of clinical signs (Fig. 1a). By day 6, 4 out of 7 mice had clinical scores of 2 or greater and all mice went on to develop full hind limb paralysis by day 8. In this particular experiment 5 out of 7 mice failed to recover from the paralysis.

[0036] Spleen cells from PLP immunized mice stimulated in vitro with antigen for 96 hours in the absence or presence of mIL-12 (20ng/ml) were also examined to determine whether they could transfer disease to naive SJL/J mice. The severity of disease following the adoptive transfer of  $30 \times 10^6$  PLP stimulated spleen cells was mild compared to that induced by an equivalent number of PLP stimulated LNC, with only 2 out of 5 mice developing complete hind limb paralysis and the remaining 3 mice displaying only mild signs of disease (Fig. 1b). Similar to the results observed with LNC, the addition of mIL-12 (20ng/ml) to the in vitro culture of spleen cells prior to transfer exacerbated subsequent disease (Fig. 1b). Mice receiving spleen cells stimulated with PLP and mIL-12 developed clinical signs of disease by day 6 and all progressed to full hind limb paralysis by day 12. The mean duration of paralysis in these mice was 5.4 days (range 2-8 days).

#### Cytokine production following in vitro stimulation of LNC with PLP and IL-12

[0037] To determine the effects of IL-12 on cytokine production during the in vitro stimulation with antigen, LNC from PLP primed mice were cultured with either PLP alone, PLP and IL-12 (20ng/ml) or PLP, IL-12 and a neutralizing anti-IFN- $\gamma$  antibody. At the end of the in vitro culture, IFN- $\gamma$  and TNF- $\alpha$  in the supernatant were measured by ELISA and the cells tested for their ability to transfer disease to naive mice. The addition of IL-12 during the in vitro stimulation of LNC with PLP resulted in a greater than 10 fold increase in IFN- $\gamma$  (5.2ng/ml control and 64ng/ml IL-12) and a two fold increase in TNF- $\alpha$  in the cell culture supernatant (Fig. 2a). The addition of a neutralizing antibody to IFN- $\gamma$  during the culture of LNC with antigen and IL-12 completely blocked IFN- $\gamma$  detection, but had no effect on the increase in TNF- $\alpha$  in the supernatants which remained approximately two fold higher relative to controls (100pg/ml controls compared to 180pg/ml with  $\alpha$ IFN- $\gamma$  antibody). Furthermore, transfer of the cells stimulated in vitro with PLP and IL-12 in the presence of a neutralizing antibody to IFN- $\gamma$  were still capable of inducing severe disease with the same kinetics and duration to that seen following the transfer of cells stimulated with PLP and IL-12 alone (Fig. 2b).

#### The effect of in vivo administration of IL-12 on disease progression

[0038] Following the transfer of  $30 \times 10^6$  PLP stimulated LNC mice were administered mIL-12 (0.3 $\mu$ g/ mouse) or saline for 3 days and the effects on the subsequent course of disease monitored. The onset and progression of disease in the controls was similar to that described above with clinical signs evident between days 6-8 after the transfer of LNC with 80% of the mice progressing to full bilateral hind limb paralysis. Peak disease in the control mice lasted approximately 3 days after which time the mice spontaneously recovered (Fig. 3a). Administration of mIL-12 (0.3 $\mu$ g/ mouse) for 3 days after the transfer of an equivalent number of primed LNC from the same in vitro cultures dramatically altered the course of disease. Although the time of onset of symptoms was only slightly earlier in the IL-12 treated mice (day 5), the subsequent progression to peak disease was accelerated with all mice displaying full hind limb paralysis by day 8. The duration of paralysis was also significantly prolonged lasting up to 14 days (range 11-14). Several mice treated with mIL-12 that developed prolonged paralysis which persisted after the controls had fully recovered were sacrificed.

[0039] In a separate experiment, the effects of in vivo administration of mIL-12 on disease severity was examined following the transfer of a suboptimal number of LNC ( $10 \times 10^6$  cells). Control mice receiving this lower number of LNC developed mild disease (Fig. 3b) with 1 out of 4 animals progressing to full hind limb paralysis and only minimal disease in the remaining 3 controls. In contrast, mice treated with mIL-12 in vivo following the transfer of  $10 \times 10^6$  LNC cells developed full clinical symptoms of disease with all mice scoring 2 or greater and 3 out of 4 mice progressing to full hind limb paralysis. The effects of mIL-12 were also apparent after the transfer of as few as  $5 \times 10^6$  LNC cells with 3 out of 5 mice reaching a score of 1. At this cell number controls showed no signs of disease (data not shown).

#### The effects of Anti-IL-12 antibody administration on the course of disease.

[0040] To determine if endogenous IL-12 plays an essential role in disease transfer, mice were treated with 200 $\mu$ g of a sheep polyclonal antibody to murine IL-12 every other day for either 6 or 12 days following the transfer of  $30 \times 10^6$  PLP stimulated LNC cells. Controls received an equal amount of sheep IgG. The onset of clinical signs in the Sheep IgG treated controls was similar to that seen in untreated mice receiving PLP stimulated LNC (day 6-7, Fig. 4a). All

control mice developed signs of disease graded 2 or greater (70% developed full paralysis). Administration of the anti-IL-12 antibody during the first 6 days after transfer did not reduce the severity of disease, however, the onset of clinical signs was delayed by approximately 7 days. These mice subsequently went on to develop disease with all mice reaching a score of 2 or greater (80% developed full paralysis) with a similar time course of recovery to control animals. To determine if this delay of disease transfer could be sustained by a longer administration of anti-IL-12 antibody, we treated mice for 12 days following adoptive transfer of PLP primed LNC. Mice treated with anti-IL-12 antibody every other day for 12 days after the transfer of PLP stimulated LNC not only showed a more sustained delay in the kinetics of disease onset but also experienced dramatically reduced clinical disease with only 2 out of 5 mice developing mild signs of disease (Fig. 4b).

## Example 2

[0041] NOD/LtJ mice (Jackson Laboratories) were treated with IL-12 to gauge the effect of the cytokine on an accepted animal model of insulin-dependent diabetes mellitus (IDDM) [Kutani et al., Adv. Immunol. 51, 1992, p. 285]. Female NOD mice spontaneously develop an IDDM-like disease with destruction of the Beta cells in the pancreas and spilling of glucose into the urine beginning around 12-14 weeks of age. In the inventor's animal facility, female NOD mice show a disease incidence of approximately 88% by 30 weeks of age.

[0042] Female NOD mice were treated with two different protocols. In Treatment A, mice were given 10, 1 or 0.1  $\mu$ g (0.5, 0.05 or 0.005 mg/kg) murine IL-12 (mIL-12) i.p. three times a week for two weeks beginning at 9-11 weeks of age. In Treatment B, mice were given 1 or 0.1  $\mu$ g mIL-12 i.p. once a week beginning at 9 weeks of age and were continued on treatment until 25 weeks of age.

[0043] Mice under Treatment A receiving all three doses showed statistically significant decreases of disease incidence, with the 10  $\mu$ g dose being most effective (17% disease incidence) (see Table 1 and Fig. 5). Mice under Treatment B receiving 1  $\mu$ g weekly showed a large decrease in disease incidence (20%), while mice receiving 0.1  $\mu$ g did not show a measurable change in disease incidence (80%) (see Table 1 and Fig. 6).



Table 1

## INCIDENCE OF DIABETES IN IL-12 TREATED NOD MICE

age	unRx	IL-12 3X/week 2 weeks*			IL-12 weekly†	
		10µg	1µg	0.1µg	1µg	0.1µg
8					0/10	0/10
9					0/10	0/10
10	0/10	0/6	1/5	0/11	0/10	0/10
11	0/20	1/6	2/5	2/11	0/10	0/10
12	1/25	1/6	2/10	2/11	0/10	0/10
13	2/25	1/6	2/10	2/11	0/10	0/10
14	3/25	2/6	2/10	2/11	0/10	1/10
15	4/25	1/6	2/10	3/11	0/10	1/10
16	5/25	1/6	2/10	3/11	0/10	4/10
17	6/25	1/6	3/10	3/11	0/10	4/10
18	6/25	1/6	3/10	4/11	0/10	7/10
19	9/25	1/6	3/10	4/11	1/10	7/10
20	12/25	1/6	4/10	5/11	1/10	7/10
21	12/25	1/6	4/10	5/11	1/10	7/10
22	14/25	1/6	5/10	5/11	1/10	7/10
23	15/25	1/6	5/10	5/11	1/10	7/10
24	16/25	1/6	5/10	5/11	1/10	7/10
25	18/25	1/6	5/10	5/11	1/10	7/10
26	20/25	1/6	5/10	5/11	2/10	7/10
27	20/25	1/6	5/10	5/11	2/10	7/10
28	21/25	1/6	5/10	5/11	2/10	7/10
29	22/25	1/6	5/10	5/11	2/10	7/10
30	22/25	1/6	5/10	5/11	2/10	8/10

\* - treatment started at 9-10 weeks of age and continued for two weeks

† - treatment started at 9 weeks of age and continued for 15 weeks

## Claims

1. Use of an IL-12 antagonist for the preparation of a pharmaceutical composition for treating in a mammalian subject an autoimmune condition.
2. The use of claim 1 wherein said IL-12 antagonist is an antibody or fragment thereof immunoreactive with IL-12.

3. The use of claim 2 wherein said antibody is a chimeric antibody or a fragment thereof.
4. The use of any one of claims 1 to 3 wherein said antagonist is to be administered in a dose of 0.05 to 25 mg/kg.
- 5 5. The use of any one of claims 1 to 3 wherein said antagonist is to be administered in a dose of 0.2 to 2 mg/kg.
6. Use of IL-12 for the preparation of a pharmaceutical composition for treating in a mammalian subject an autoimmune condition.
- 10 7. The use of claim 6 wherein said IL-12 is to be administered at 0.001 to 1000 µg/kg.
8. The use of claim 6 wherein said IL-12 is to be administered at 0.01 to 100 µg/kg.
- 15 9. The use of any one of claims 1 to 8 wherein said autoimmune condition is promoted by an increase in levels of a cytokine selected from TNF-α or IFN-γ.
10. The use of claim 9 wherein said autoimmune condition is selected from multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus and autoimmune inflammatory eye disease.
- 20 11. The use of any one of claims 1 to 5, 9 or 10 wherein said autoimmune condition is multiple sclerosis.
12. The use of any one of claims 1 to 5, 9 or 10 wherein said autoimmune condition is rheumatoid arthritis.
- 25 13. The use of any one of claims 6 to 10, wherein said autoimmune condition is insulin-dependent diabetes mellitus.
14. The use of any one of claims 1 to 13 wherein said pharmaceutical composition comprises a pharmaceutically acceptable carrier.
- 30 15. The use of any one of claims 1 to 14 wherein said pharmaceutical composition contains 0.1 microgram to 1 milligram per milliliter of the IL-12 antagonist or IL-12.

#### Patentansprüche

- 35 1. Verwendung eines IL-12-Antagonisten für die Herstellung einer pharmazeutischen Zusammensetzung für die Behandlung einer Autoimmunerkrankung in einem Säugetier.
- 40 2. Verwendung nach Anspruch 1, wobei der IL-12-Antagonist ein Antikörper oder ein Fragment davon mit Immunreaktivität gegenüber IL-12 ist.
3. Verwendung nach Anspruch 2, wobei der Antikörper ein chimärer Antikörper oder ein Fragment davon ist.
- 45 4. Verwendung nach einem der Ansprüche 1 bis 3, wobei der Antagonist in einer Dosis von 0,05 bis 25 mg/kg zu verabreichen ist.
5. Verwendung nach einem der Ansprüche 1 bis 3, wobei der Antagonist in einer Dosis von 0,2 bis 2 mg/kg zu verabreichen ist.
- 50 6. Verwendung von IL-12 für die Herstellung einer pharmazeutischen Zusammensetzung für die Behandlung einer Autoimmunerkrankung in einem Säugetier.
7. Verwendung nach Anspruch 6, wobei das IL-12 in einer Menge von 0,001 bis 1000 µg/kg zu verabreichen ist.
- 55 8. Verwendung nach Anspruch 6, wobei das IL-12 in einer Menge von 0,01 bis 100 µg/kg zu verabreichen ist.
9. Verwendung nach einem der Ansprüche 1 bis 8, wobei die Autoimmunerkrankung gefördert wird durch eine Erhöhung der Spiegel eines Zytokins, das ausgewählt ist aus TNF-α oder IFN-γ.

10. Verwendung nach Anspruch 9, wobei die Autoimmunerkrankung ausgewählt ist aus Multipler Sklerose, systemischem Lupus erythematoses, rheumatoider Arthritis, autoimmuner Lungenentzündung, Guillain-Barré-Syndrom, Autoimmunthyreoiditis, insulin-abhängigem Diabetes mellitus und autoimmuner entzündlicher Augenerkrankung.

11. Verwendung nach einem der Ansprüche 1 bis 5, 9 oder 10, wobei die Autoimmunerkrankung Multiple Sklerose ist.

12. Verwendung nach einem der Ansprüche 1 bis 5, 9 oder 10, wobei die Autoimmunerkrankung rheumatoide Arthritis ist.

13. Verwendung nach einem der Ansprüche 6 bis 10, wobei die Autoimmunerkrankung insulin-abhängiger Diabetes mellitus ist.

14. Verwendung nach einem der Ansprüche 1 bis 13, wobei die pharmazeutische Zusammensetzung einen pharmazeutisch verträglichen Träger umfasst.

15. Verwendung nach einem der Ansprüche 1 bis 14, wobei die pharmazeutische Zusammensetzung 0,1 Mikrogramm bis 1 Milligramm pro Milliliter des IL-12-Antagonisten oder IL-12 enthält.

## Revendications

1. Utilisation d'un antagoniste d'IL-12 pour la préparation d'une composition pharmaceutique pour traiter une maladie autoimmune chez un sujet mammifère.

2. Utilisation selon la revendication 1, où ledit antagoniste d'IL-12 est un anticorps ou un fragment de celui-ci immunoréactif avec IL-12.

3. Utilisation selon la revendication 2, où ledit anticorps est un anticorps chimère ou un fragment de celui-ci.

4. Utilisation selon l'une quelconque des revendications 1 à 3, où ledit antagoniste doit être administré en une dose de 0,05 à 25 mg/kg.

5. Utilisation selon l'une quelconque des revendications 1 à 3, où ledit antagoniste doit être administré en une dose de 0,2 à 2 mg/kg.

6. Utilisation d'IL-12 pour la préparation d'une composition pharmaceutique pour traiter une maladie autoimmune chez un sujet mammifère.

7. Utilisation selon la revendication 6, où ladite IL-12 doit être administrée à raison de 0,001 à 1 000 µg/kg.

8. Utilisation selon la revendication 6, où ladite IL-12 doit être administrée à raison de 0,01 à 100 µg/kg.

9. Utilisation selon l'une quelconque des revendications 1 à 8, où ladite maladie autoimmune est favorisée par une augmentation des niveaux d'une cytokine choisie parmi TNF-α et IFN-γ.

10. Utilisation selon la revendication 9, où ladite maladie autoimmune est choisie parmi la sclérose en plaques, le lupus érythémateux aigu disséminé, la polyarthrite rhumatoïde, l'inflammation pulmonaire autoimmune, le syndrome de Guillain-Barré, la thyroïdite autoimmune, le diabète sucré insulino-dépendant et la maladie oculaire inflammatoire autoimmune.

11. Utilisation selon l'une quelconque des revendications 1 à 5, 9 ou 10, où ladite maladie autoimmune est la sclérose en plaques.

12. Utilisation selon l'une quelconque des revendications 1 à 5, 9 ou 10, où ladite maladie autoimmune est la polyarthrite rhumatoïde.

13. Utilisation selon l'une quelconque des revendications 6 à 10, où ladite maladie autoimmune est le diabète sucré insulino-dépendant.

**14.** Utilisation selon l'une quelconque des revendications 1 à 13, où ladite composition pharmaceutique comprend un support pharmaceutiquement acceptable.

**15.** Utilisation selon l'une quelconque des revendications 1 à 14, où ladite composition pharmaceutique contient 0,1 µg à 1 mg/ml d'antagoniste d'IL-12 ou d'IL-12.

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Fig 1

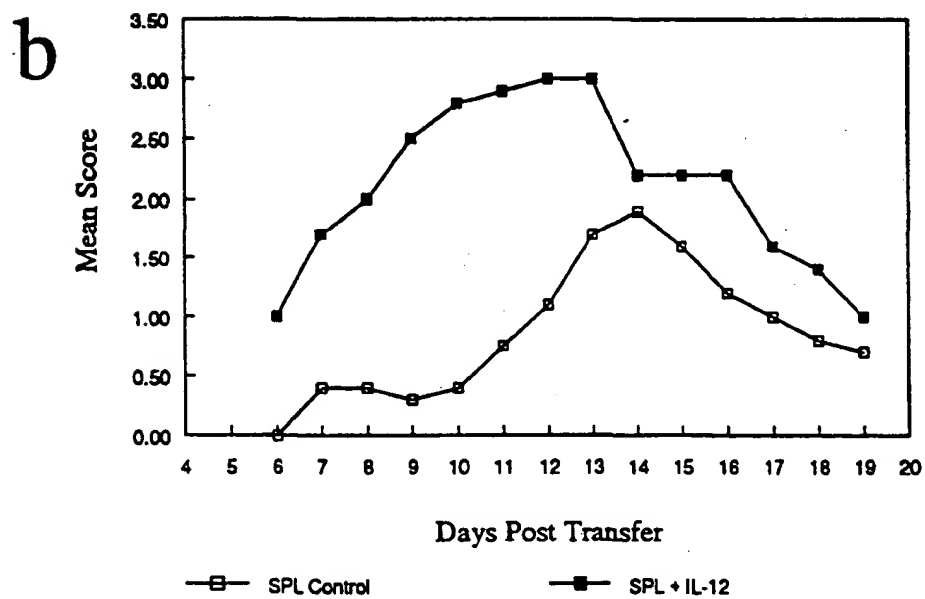
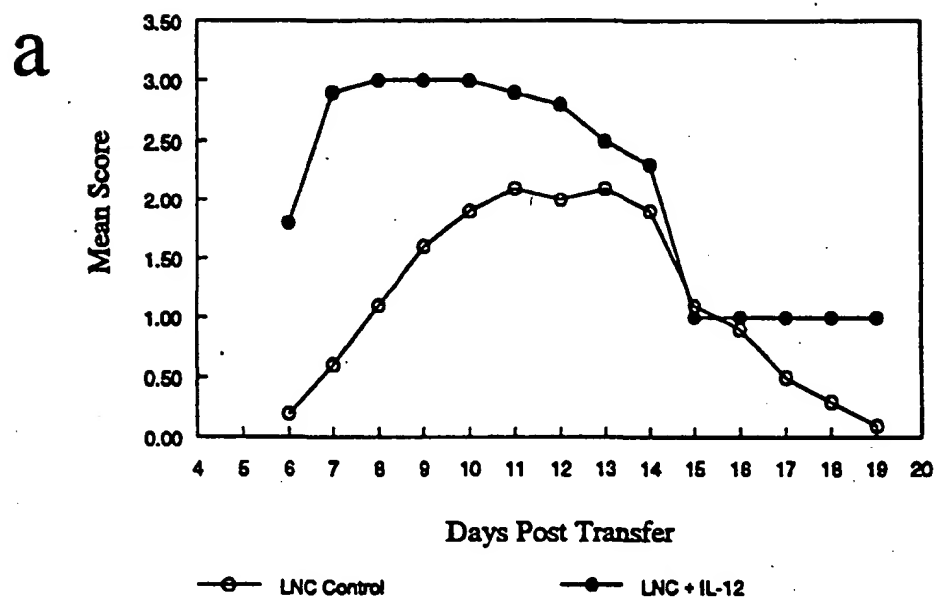


Fig 2

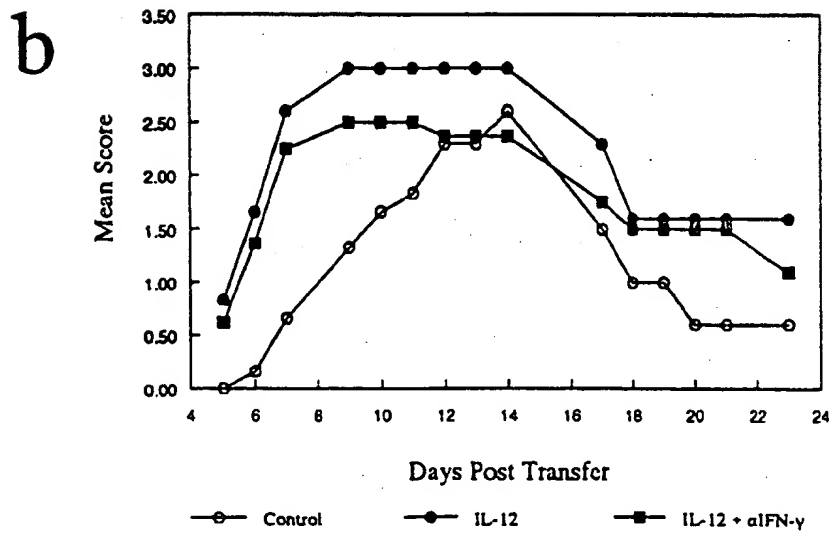
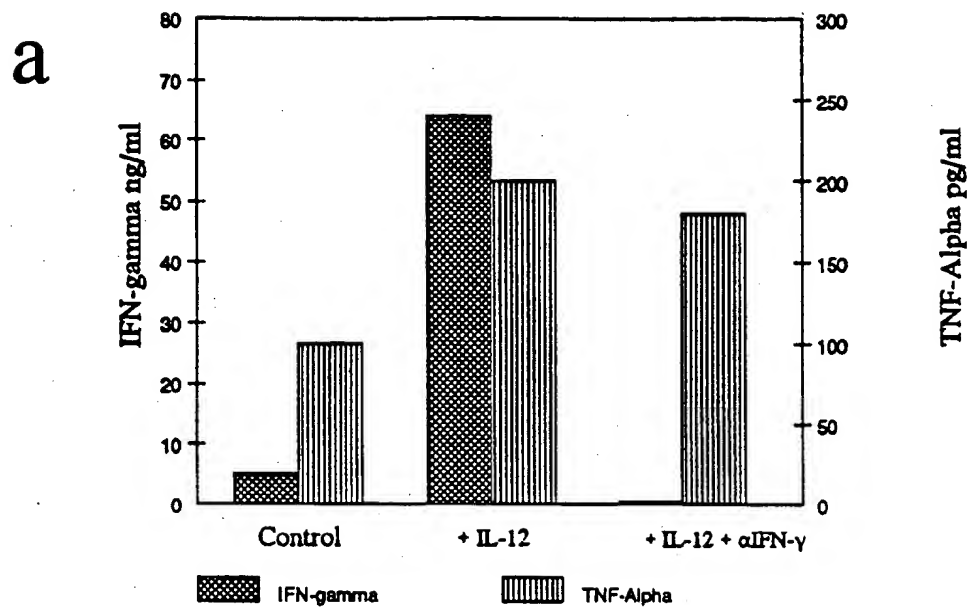


Fig 3

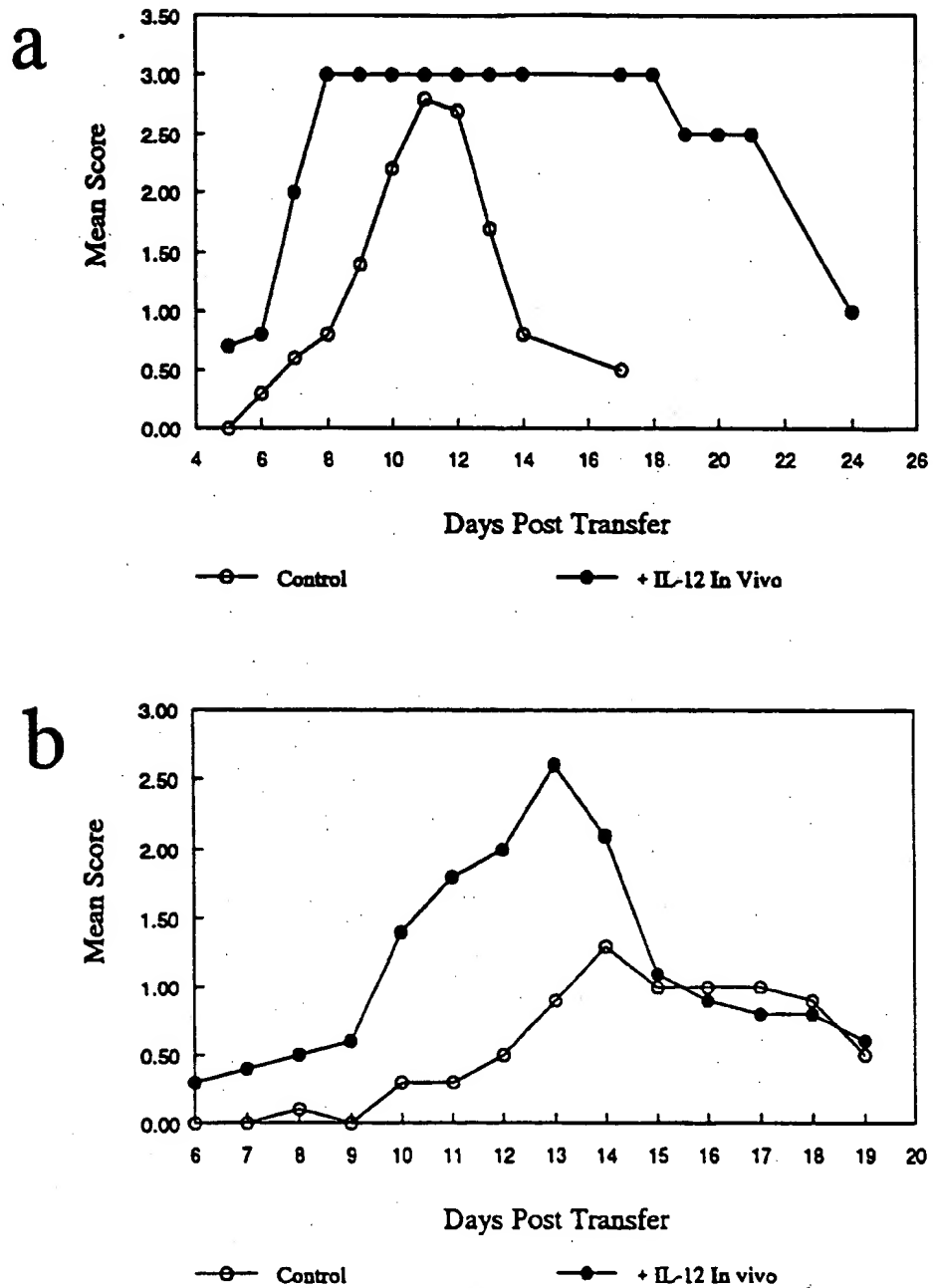


Fig 4

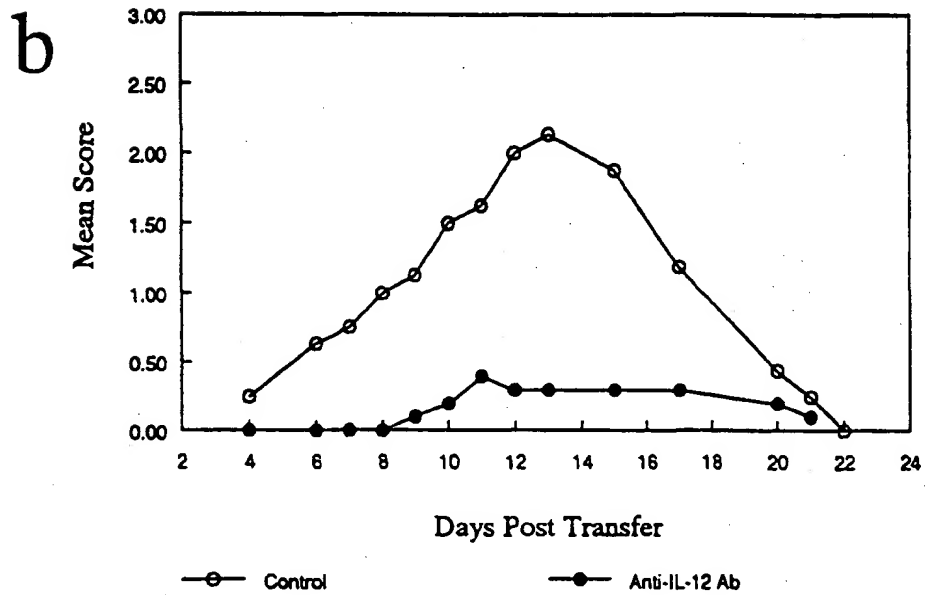
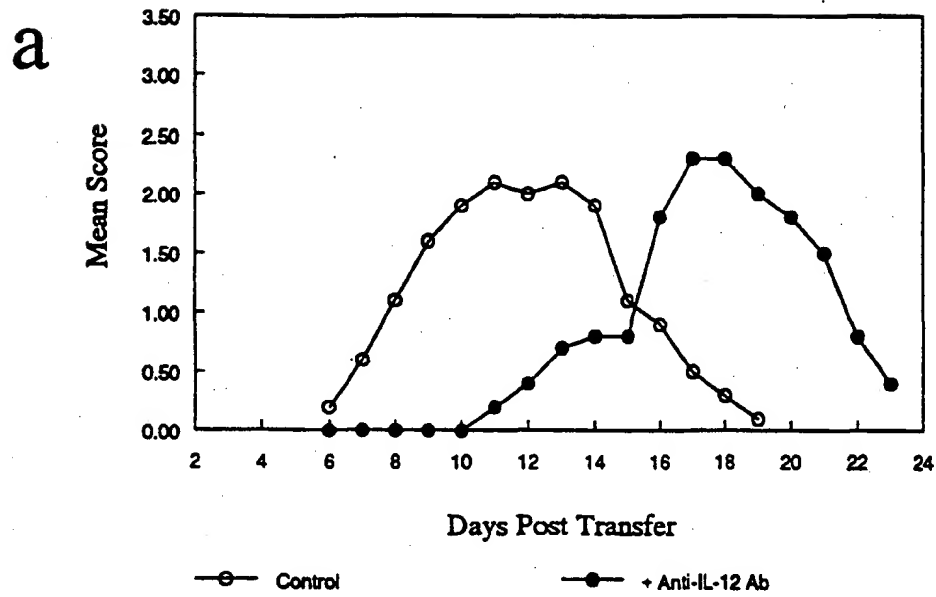




Fig. 5

Protocol A

IL-12/NOD disease incidence combined data

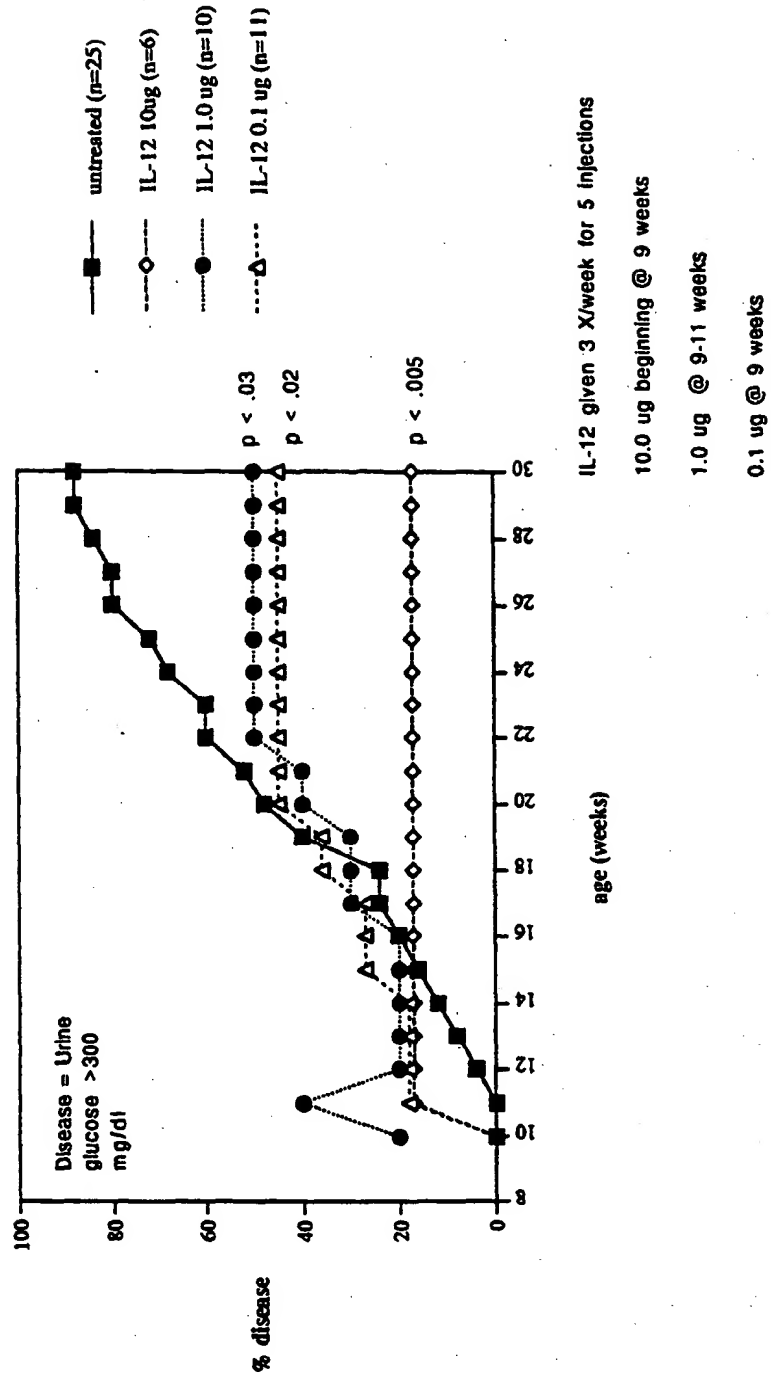


Fig. 6

